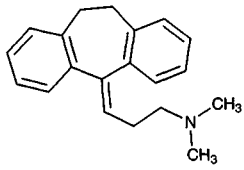
Amitriptyline



Molecular formula: $C_{20}H_{23}N$
Molecular weight: 277.41
CAS Registry No.: 50-48-6, 549-18-8 (HCl)
Merck Index: 511
Lednicer No.: 1 151, 404

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 100 μL 400 μg/mL IS and 2 mL 500 mM NaOH, vortex briefly, add 4 mL heptane:isoamyl alcohol 98.5:1.5 and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL heptane:isoamyl alcohol 98.5:1.5 to extraction sample, mix. Combine the organic layers and extract them with 2 mL 50 mM sulfuric acid. Make the acid layer alkaline with 1 mL 1.0 M pH 9.0 carbonate/bicarbonate buffer and mix with 2 mL toluene:isoamyl alcohol 85:15 for 15 min. Evaporate the organic layer to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II OD
Mobile phase: MeCN:pH 3 phosphate buffer:n-nonylamine 40-50:60:0.12
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.48
Internal standard: doxepin (2.99)

OTHER SUBSTANCES

Extracted: nortriptyline

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)

Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 11.53
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, clomipramine, desipramine, fluoxetine, imipramine maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid
Interfering: levomepromazine

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 30 mg Oasis HLB SPE cartridge with 1 mL MeOH and 1 mL water. Acidify (?) mL serum with 20 μL phosphoric acid, vortex for 5 s, add to the SPE cartridge, wash with 1 mL MeOH :water 5:95, elute with 1 mL MeOH. Evaporate the eluate to dryness at 40° under a stream of nitrogen. Reconstitute the residue with 200 μL MeOH:20 mM pH 7 phosphate buffer 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 Sentry
Column: 150 × 3.9 5 μm Symmetry C18 (Waters)
Mobile phase: MeOH:20 mM pH 7 potassium phosphate 70:30
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 21
Internal standard: nordoxepin (4.9)

OTHER SUBSTANCES
Simultaneous: metabolites, doxepin, nortriptyline

KEY WORDS
pig; serum; SPE

REFERENCE
Cheng,Y.-F.; Phillips,D.J.; Neue,U.; Bean,L. Solid-phase extraction for the determination of tricyclic antidepressants in serum using a novel polymeric extraction sorbent, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2461–2473.

SAMPLE
Matrix: blood, gastric contents, tissue
Sample preparation: Blood. Mix 200 μL blood with 200 μL pH 11 borate buffer and 40 μL aqueous trimipramine solution. Extract with 800 μL hexane:2-butanol 98:2, centrifuge at 740 g for 10 min, evaporate the organic phase under vacuum at 40° (Buchler Vortex Evaporator, USA). Dissolve the residue in 100 μL mobile phase, inject an aliquot. Tissue, gastric contents. Homogenize tissue in pH 11 borate buffer to a final concentration 200 mg tissue/mL homogenate (Ultraturrax T5 homogenizer, IKA, Germany), dilute gastric contents 1:9 with water. Mix 200 μL tissue homogenate or diluted gastric contents with 40 μL aqueous trimipramine solution. Extract with 800 μL hexane:2-butanol 98:2, centrifuge at 740 g for 10 min, evaporate the organic phase under vacuum at 40° (Buchler Vortex Evaporator, USA). Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was saturated aqueous sodium tetraborate adjusted to pH 11 with 6 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-Si
Mobile phase: MeCN:25 mM ammonium acetate 90:10
Flow rate: 3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Internal standard: trimipramine maleate
Limit of detection: 40 nmol/kg (blood), 200 nmol/kg (tissue)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood; bone marrow; kidney; liver; lung; muscle; pharmacokinetics; pig; vitreous humor

REFERENCE
Hilberg,T.; Ripel,.; Smith,A.J.; Slordal,L.; Morland,J.; Bjorneboe,A. Postmortem amitriptyline pharmacokinetics in pigs after oral and intravenous routes of administration, *J.Forensic Sci.*, **1998**, *43*, 380–387.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 1 μg/mL desipramine and 100 μL 5 M NaOH for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 30:70 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 10.5
Internal standard: desipramine (6.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: nortriptyline
Simultaneous: ketoconazole
Noninterfering: diazepam, furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin

KEY WORDS
human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15 000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 16.2 (A), 28.5 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES
Extracted: amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 15.878

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 Supelco C18
Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

CHROMATOGRAM

Retention time: 11.5
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)

OTHER SUBSTANCES

Extracted: citalopram, nortriptyline
Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol
Noninterfering: benzodiazepines
Interfering: didesmethylclomipramine, levomepromazine

KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites, *J.Chromatogr.B*, **1996**, *675*, 83–88.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 239

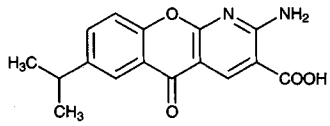
OTHER SUBSTANCES

Also analyzed: chlorpromazine, clomipramine, promazine, promethazine, thymol

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Amlexanox



Molecular formula: $C_{16}H_{14}N_2O_4$
Molecular weight: 298.30
CAS Registry No.: 68302-57-8
Merck Index: 515

SAMPLE

Matrix: tissue
Sample preparation: Cut eye tissue in small pieces, add 5 mL MeoH, shake for 30 min, let stand overnight, centrifuge at 3000 rpm for 10 min. Remove 4 mL of the supernatant and evaporate it to dryness under vacuum, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: Schim-pack C18
Mobile phase: MeCN:50 mM pH 8.0 NaH_2PO_4 25:73
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 350 em 402

CHROMATOGRAM

Internal standard: 4-methylumbelliferone
Limit of detection: < 4000 ng/g

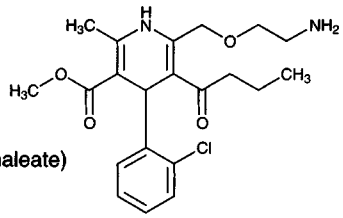
KEY WORDS

rat; eye

REFERENCE

Rankov,G.; Sasaki,K.; Fukuda,M. Pharmacodynamics of Amlexanox (AA-673) in normal and anaphylactic rat conjunctiva and its effect on histamine concentration, *Ophthalmic Res.*, **1990**, *22*, 359–364.

Amlodipine



Molecular formula: $C_{20}H_{25}ClN_2O_5$
Molecular weight: 408.88
CAS Registry No.: 88150-42-9, 111470-99-6 (besylate), 88150-47-4 (maleate)
Merck Index: 516
Lednicer No.: 4 108

SAMPLE

Matrix: blood

Sample preparation: Mix 5.0 mL plasma with 250 ng chloroamlodipine, add 250 μL 1 M sodium hydroxide and 5 mL chloroform, shake for 10 min, centrifuge at 3500 g. Dry the organic layer in a vacuum centrifuge, wash the tube twice with 500 μL chloroform and dry again. Dissolve the extract in 70 μL MeOH:pH 4.5 acetic buffer 60:40, inject a 50 μL aliquot. (For added sensitivity each eluting enantiomer may be trapped separately on 20 × 4.6 5 μm Supelcosil LC8 columns and eluted from these columns with MeCN:10 mM pH 4.5 acetate buffer 45:55 and chromatographed on 150 × 4.6 4 μm Symmetry C 8 columns.)

HPLC VARIABLES

Column: 150 × 4 Chiral AGP (ChromTech, Haegerstern, Sweden)
Mobile phase: 1-Propanol:10 mM pH 4.5 acetate buffer 1:99
Column temperature: 30
Flow rate: 0.9
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 11. 5 (R-(+)-), 15.4 (S-(-)-)
Internal standard: chloroamlodipine (29.2 (R-(+)), 36.6 (S-(-))

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Luksa,J.; Josic,D.; Kremser,M.; Kopitar,Z.; Milutinovic,S. Pharmacokinetic behaviour of R-(+)- and S-(-)-amlodipine after single enantiomer administration, *J.Chromatogr.B*, **1997**, *703*, 185–193.

SAMPLE

Matrix: blood

Sample preparation: 5 mL Plasma + 250 ng chloramlodipine + 250 μL 1 M NaOH + 5 mL chloroform, shake for 10 min, centrifuge at 3500 g. Evaporate the organic layer to dryness in a vacuum centrifuge, add 500 μL chloroform to the residue, evaporate to dryness, add 500 μL chloroform to the residue, evaporate to dryness, reconstitute with 70 μL MeOH:pH 4.5 acetate buffer 60:40, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, collect each enantiomer on SEPARATE columns B. After 40 min elute the column B containing R-amlodipine with mobile phase B onto column C, monitor the effluent from column C, later elute the column B containing S-amlodipine with mobile phase B onto column C, monitor the effluent from column C.

HPLC VARIABLES

Column: A 150 × 4 Chiral AGP (ChromTech); B 20 × 4.6 Supelcosil LC-8; C 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: A n-Propanol:10 mM pH 4.5 acetate buffer 1:99; B MeCN:10 mM pH 4.5 acetate buffer 45:55

Column temperature: 30 (column C only)
Flow rate: 0.9
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 42.9 (R-(+)), 58.3 (S-(-)) [after start of analysis]
Internal standard: chloramlodipine (Lek)
Limit of detection: <5 ng/mL

KEY WORDS
chiral; plasma; column-switching

REFERENCE
Luksa,J.; Josic,D.; Podobnik,B.; Furlan,B.; Kremser,M. Semi-preparative chromatographic purification of the enantiomers of S-(-)-amlodipine and R-(+)-amlodipine, *J.Chromatogr.B*, **1997**, *693*, 367–375.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.093

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Inject an 800 μL aliquot of a 1.3 mg/mL solution.

HPLC VARIABLES
Column: 150 × 10 Chiral AGP (ChromTech, Sweden)

Mobile phase: n-Propanol:10 mM pH 4.5 acetate buffer 1:99
Flow rate: 4
Injection volume: 800
Detector: UV 240

CHROMATOGRAM

Retention time: 35 (R-(+)), 48 (S-(-))

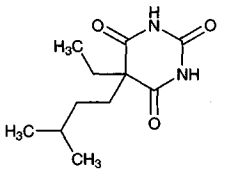
KEY WORDS

chiral; semi-preparative

REFERENCE

Luksa,J.; Josic,D.; Podobnik,B.; Furlan,B.; Kremser,M. Semi-preparative chromatographic purification of the enantiomers of S-(-)-amlodipine and R-(+)-amlodipine, *J.Chromatogr.B*, **1997**, *693*, 367–375.

Amobarbital



Molecular formula: $C_{11}H_{18}N_2O_3$
Molecular weight: 226.28
CAS Registry No.: 57-43-2, 64-43-7 (sodium salt)
Merck Index: 607
Lednicer No.: 1 268

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 23.0
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)

Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 6.05
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 µL plasma then 50 µL 10 µg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 µL mobile phase, inject a 15 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 9.64
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.617

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk quantities. Dissolve 25 mg bulk quantities in 25 mL MeCN, dilute 1:10 with MeCN, inject an aliquot. Tablets. Crush tablet, add 46 mg to 10 mL MeCN, shake for 24 h, dilute 1 mL clear supernatant 1:10 with MeCN, inject an aliquot. Capsules. Powder capsule, add 35 mg to 1 mL 10% sulfuric acid, shake, make up to 10 mL with MeCN, shake for 24 h, dilute 1 mL clear supernatant 1:10 with MeCN, inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 2 37-53 μm Whatman pellicular ODS
Column: 250 × 4.6 5 μm Econosphere C18 (Alltech)
Mobile phase: MeCN:water 30:70
Flow rate: 1.2
Detector: UV 198

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
tablets; capsules

REFERENCE
Soine,W.H.; Graham,R.M.; Soine,P.J. Identification of 5-ethyl-5-(2-methylbutyl)barbituric acid as an impurity of manufacture in amobarbital, *J.Pharm.Sci.*, **1992**, *81*, 362–364.

SAMPLE
Matrix: saliva
Sample preparation: Saliva sample obtained by chewing on 100 sq. cm Parafilm, centrifuge at 3000 g for 5 min. Remove a 1 mL aliquot of the supernatant and add it to 100 μL 5 μg/mL hexobarbital solution, add the mixture to a 3 mL octadecyl Baker-10 SPE cartridge, wash with three 3 mL portions of water, elute with 800 μL MeOH, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 30 × 4 5 μm Develosil ODS-5 (Nomura)
Column: 150 × 4 5 μm Develosil ODS-5 (Nomura)
Mobile phase: MeOH:water 50:50
Flow rate: 0.8
Injection volume: 50
Detector: UV 240 following a 150 × 3 sulfonated hollow-fiber membrane reactor (Dionex AFS-2) which was surrounded by 50 mM pH 10.2 ammonium hydroxide solution

CHROMATOGRAM
Retention time: 14
Internal standard: hexobarbital (10)
Limit of detection: 0.5-2.5 ng

OTHER SUBSTANCES
Simultaneous: barbital, phenobarbital

KEY WORDS
SPE; post-column reaction

REFERENCE
Haginaka,J.; Wakai,J. Liquid chromatographic determination of barbiturates using a hollow-fibre membrane for postcolumn pH modification, *J.Chromatogr.*, **1987**, *390*, 421–428.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 8
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: barbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, pentobarbital, phenobarbital, secobarbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate a solution in water, MeOH, or diethyl ether to dryness, add a 3-fold molar excess of triethylamine, add 0.5-3 mL MeCN, add a 3-fold molar excess of N-chloromethyl-4-nitrophthalimide, heat at 60° for 1 h, inject an aliquot. (Preparation of N-chloromethyl-4-nitrophthalimide is as follows. Suspend 130 g 4-nitrophthalimide in 80 mL 40% formaldehyde solution, add 200 mL water, reflux for 4 h, filter while hot, N-(hydroxymethyl-4-nitrophthalimide crystallizes on cooling (cf. J. Am. Chem. Soc. 1922, 44, 817). Mix a suspension of 2.26 g N-hydroxymethyl-4-nitrophthalimide in 10-15 mL ether with a suspension of 2.1 g phosphorus pentachloride in 10-15 mL ether, after 10 min heat on a water bath, cool in an ice-salt mixture, add ice-water dropwise with shaking, filter to obtain N-chloromethyl-4-nitrophthalimide, dry under vacuum (cf. Chem. Ber. 1959, 9, 1258).)

HPLC VARIABLES
Column: 7 μm LiChrosorb RP8
Mobile phase: MeCN:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 4 ng

OTHER SUBSTANCES
Extracted: secobarbital
Simultaneous: cyclobarbital, methylphenobarbital, phenobarbital

KEY WORDS
derivatization

REFERENCE
Lindner,W.; Santi,W. N-chloromethylphthalimides as derivatization reagents for high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *176*, 55–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, methohexital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 1.95

OTHER SUBSTANCES
Simultaneous: aprobarbital, pentobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE
Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 2 μBondapak C18
Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid
Flow rate: 0.27
Injection volume: 5
Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: butethal, butabarbital, talbutal, butalbital, pentobarbital

KEY WORDS
mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.94 (A), 5.59 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, pheno-

barbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 200 μL Urine + 1 mL saturated ammonium sulfate, extract with 2 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

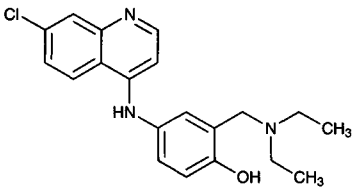
HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltech C18
Mobile phase: MeCN:25 mM pH 6.5 sodium phosphate buffer 20:80
Flow rate: 1.4
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 41.5

OTHER SUBSTANCES
Extracted: metabolites, conjugates

REFERENCE
Soine,W.H.; Soine,P.J.; Overton,B.W.; Garrettson,L.K. Product enantioselectivity in the N-glucosylation of amobarbital, *Drug Metab.Dispos.*, **1986**, *14*, 619–621.

Amodiaquin



Molecular formula: $C_{20}H_{22}ClN_3O$
Molecular weight: 355.87
CAS Registry No.: 86-42-0, 6398-98-7 (dihydrochloride dihydrate)
Merck Index: 609
Lednicer No.: 4 140

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 4.62
Limit of detection: <120 ng/mL

KEY WORDS

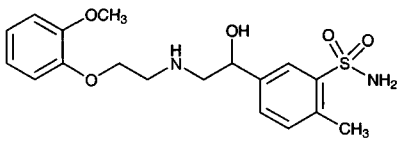
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Amosulalol



Molecular formula: $C_{18}H_{24}N_2O_5S$
Molecular weight: 380.47
CAS Registry No.: 85320-68-9
Merck Index: 614

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL saturated sodium bicarbonate solution and 100 μL water to 1.5 mL plasma, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 2.5 mL 400 mM HCl. Shake, centrifuge, and discard the organic layer. Add 2 mL saturated sodium bicarbonate solution to the aqueous layer and extract again with 5 mL ethyl acetate. Evaporate the organic layer to dryness under reduced pressure, reconstitute the residue with 50 μL 100 mM sodium bicarbonate. Add 100 μL 5 mg/mL dansyl chloride in acetone. Heat at 35° for 90 min. Add 5 mL distilled water and extract with 5 mL diethyl ether. Evaporate the organic layer to dryness at 45°, reconstitute the residue with 60 μL mobile phase. Inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 Nucleosil SI100-5 (Chemco, Japan)
Mobile phase: MeOH:benzene 1:100 (Caution! Benzene is a carcinogen!)
Injection volume: 20-50
Detector: F ex 352 em 500

KEY WORDS

amosulalol is IS; plasma; human; rat; dog; normal phase

REFERENCE

Matsushima,H.; Kamimura,H.; Soeishi,Y.; Watanabe,T.; Higuchi,S.; Tsunoo,M. Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans, *Drug Metab.Dispos.*, **1998**, *26*, 240–245.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL 0.5 μg/mL IS in water + 0.5 g sodium bicarbonate + 4 mL ethyl acetate, extract, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 3 mg/mL sodium bicarbonate in water and 200 μL 500 μg/mL 5-di-n-butylaminonaphthalene-1-sulfonyl chloride (Bans-Cl) in acetone, heat at 45° for 90 min, cool, add 4 mL diethyl ether, wash mixture with 3 mL water for 10 s. Remove the organic layer and evaporate it to dryness at 40-50°, reconstitute the residue in 100 μL benzene (CAUTION! Benzene is a carcinogen!), inject a 5-10 μL aliquot. (Derivatization can also be performed with 5-dimethylaminonaphthalene-1-sulfonyl chloride (Dans-Cl), F ex 250 em 505, retention time of derivative 4.1 min.)

HPLC VARIABLES

Column: 150 × 4 5 μm LiChrosorb SI-60
Mobile phase: Benzene:MeOH 50:1 (CAUTION! Benzene is a carcinogen)
Flow rate: 2
Injection volume: 5-10
Detector: F ex 356 em 500

CHROMATOGRAM

Retention time: 2.9

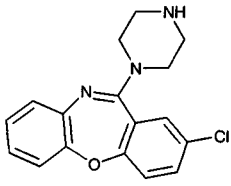
Internal standard: 5-[1-hydroxy-2-[2-(o-methoxyphenoxy)ethylamino]ethyl]-2-methoxybenzenesulfonamide (4.0)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; normal phase; derivatization; dog; pharmacokinetics

REFERENCE

Kamimura,H.; Sasaki,H.; Kawamura,S. Determination of the α,β-adrenoceptor blocker YM-09538 in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1981**, *225*, 115–121.

Amoxapine



Molecular formula: $C_{17}H_{16}ClN_3O$
Molecular weight: 313.79
CAS Registry No.: 14028-44-5
Merck Index: 616
Lednicer No.: 2 428

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum + 50 µL 100 mM HCl + 400 µL 1 M NaOH + 6 mL ethyl acetate, shake for 15 min, centrifuge at 4000 g for 10 min. Transfer top organic layer to another tube and re-extract the analyte with 3 mL 50 mM HCl. Evaporate organic layer in water bath under nitrogen at 45°. Dissolve the residue in 120 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: Perisorb RP-8 (Upchurch)
Column: 100 × 4.6 3 µm Supelco C8-DB
Mobile phase: MeCN:MeOH:buffer 20:18:62 (Buffer was 50 mM sodium monobasic phosphate, contains 2.5 mL/L triethylamine, pH adjusted to 2.7 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 30
Detector: UV 230

CHROMATOGRAM

Retention time: 14.3
Internal standard: amoxapine

OTHER SUBSTANCES

Extracted: metabolites, clozapine
Simultaneous: amitryptiline, atenolol, bupropion, cogentin, desipramine, diazepam, doxepin, fluoxetine, haloperidol, imipramine, loxapine, medazepam, nortryptiline, oxazepam, paroxetine, phenytoin, propranolol, sertraline, thiothixene, trazadone, trifluoperazine, valproic acid, verapamil
Interfering: carbamazepine, desmethylsertraline

KEY WORDS

serum; amoxapine is IS

REFERENCE

Hariharan,U.; Hariharan,M.; Naickar,J.S.; Tandon,R. Determination of clozapine and its two major metabolites in human serum by liquid chromatography using ultraviolet detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 2409–2417.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 µL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 µL aliquot of the aqueous phase (J.Liq.Chromatogr. 1981, 4, 849).

HPLC VARIABLES

Guard column: 23 × 3.9 Bondapak/Corasil C 18

Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: clomipramine (8.5)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amoxapine, amitriptyline, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine
Interfering: nortriptyline

KEY WORDS
plasma

REFERENCE
Wong,S.H.Y.; Waugh,S.W. Determination of the antidepressants maprotiline and amoxapine, and their metabolites, in plasma by liquid chromatography, *Clin.Chem.*, **1983**, *29*, 314–318.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, clomipramine, doxepin, desipramine, imipramine, maprotiline, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE

Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL 2-hydroxydesmethylimipramine, mix, add 300 μL 2 M pH 9.7 carbonate buffer, mix, add 4 mL heptane:isopentyl alcohol 93:7, shake mechanically for 20 min, centrifuge at 1600 g for 5 min. Remove the organic layer and add it to 250 μL 7 mM orthophosphoric acid, vortex vigorously, centrifuge at 1600 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb C6

Mobile phase: 105 μM nonylamine in MeCN:5 mM KH_2PO_4 + 14 mM orthophosphoric acid 23:77

Column temperature: 35

Flow rate: 2.2

Injection volume: 100

Detector: UV 210

CHROMATOGRAM

Retention time: 22

Internal standard: 2-hydroxydesmethylimipramine

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, loxapine

Simultaneous: propranolol, doxepin, desmethylimipramine, haloperidol, protriptyline, imipramine, amitriptyline

Noninterfering: chlorpromazine, clomipramine, maprotiline, nortriptyline, thioridazine, trimipramine, trifluoperazine

KEY WORDS

plasma

REFERENCE

Cheung,S.W.; Tang,S.W.; Remington,G. Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 213–221.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 μg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 μL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 11.18
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, fenfluramine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 75 μL MeCN containing 4 mg/mL loxapine + 2 mL 250 mM NaOH + 400 μL isoamyl alcohol, vortex vigorously, let stand for 5 min, add 10 mL heptane, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Remove the upper heptane layer and add it to 1 mL 100 mM pH 3 glycylglycine buffer, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Discard the heptane layer, add 1 mL 250 mM NaOH to the aqueous layer, add 5 mL n-pentane, shake for 1 h, centrifuge at 2000 g for 30 min. Remove the organic layer and evaporate it to dryness under educed pressure, reconstitute the residue in 70 μL mobile phase, vortex vigorously, centrifuge at 2000 g for 2-3 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 μm pellicular silica (CEL Associates, Houston)
Column: 100 × 6 3 μm silica 80 Å (CEL Associates, Houston)
Mobile phase: MeCN:buffer 20:80 containing 21 mM n-nonylamine, pH 7.4-7.8 (Buffer was 25 mM Na_2HPO_4 adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1.6
Injection volume: 50
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 9.63
Internal standard: loxapine (22.68)

OTHER SUBSTANCES
Extracted: doxepin
Simultaneous: amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, imipramine, mianserin, nortriptyline, thioridazine, trimipramine
Noninterfering: diazepam

KEY WORDS
serum; amoxapine is IS

REFERENCE
Adamczyk,M.; Fishpaugh,J.R.; Harrington,C. Quantitative determination of *E*- and *Z*-doxepin and *E*- and *Z*-desmethyldoxepin by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 371–376.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 299

CHROMATOGRAM
Retention time: 7.57
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; tri-

fluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 6.61
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: fluoxetine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15 000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)

Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40

Flow rate: 0.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5.6 (A), 7.8 (B)

Internal standard: diazepam (4.4, A)

Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, clomipramine, dothiepin, doxepin, imipramine, melitracen, mianserin, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

Interfering: desipramine, maprotiline

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 14.187

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: < 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, carbamazepine, imipramine, nortriptyline
Also analyzed: doxepin, desipramine, protriptyline, cyclobenzaprine, maprotiline

KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.40 (A), 5.55 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

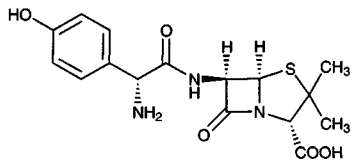
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Amoxicillin



Molecular formula: $C_{16}H_{19}N_3O_5S$
Molecular weight: 365.41
CAS Registry No.: 26787-78-0 (anhydrous), 61336-70-7 (trihydrate)
Lednicer No.: 4 179, 180

SAMPLE

Matrix: blood
Sample preparation: Vortex plasma sample for 15 s, centrifuge at 4000 rpm for 10 min at 4 °. 250 µL Plasma + 250 µL 5 µg/mL cefadroxil in 100 mM pH 2.5 KH_2PO_4, centrifuge at 4800 g for 30 min. Inject a 150 µL aliquot of the clear supernatant onto column A and elute to waste with mobile phase B, after 15 min elute the contents of column A onto column B with mobile phase A, after 5 min remove column A from the circuit, elute column B with mobile phase A, monitor effluent from column B. Re-equilibrate column A with mobile phase B.

HPLC VARIABLES

Column: A 40 × 4.6 10 µm Nucleosil 100 C18; B 250 × 4.6 5 µm Spherisorb ODS II
Mobile phase: A MeOH:10 mM sodium heptanesulfonate and 30 mM NaH_2PO_4 25:75 adjusted to pH 2.5 with phosphoric acid; B MeOH:10 mM sodium heptanesulfonate and 30 mM NaH_2PO_4 8:92 adjusted to pH 2.5 with phosphoric acid
Flow rate: 1.5
Injection volume: 150
Detector: UV 230

CHROMATOGRAM

Retention time: 34.8-38.8
Internal standard: cefadroxil (31.8-32.8)
Limit of quantitation: 50.1 ng/mL

KEY WORDS

plasma; column-switching; pharmacokinetics

REFERENCE

Muth,P.; Metz,R.; Beck,H.; Bolten,W.W.; Vergin,H. Improved high-performance liquid chromatographic determination of amoxicillin in human plasma by means of column switching, *J.Chromatogr.A*, **1996**, *729*, 259–266.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.067

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 12.5 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 32:68 (Buffer was 15 mM phosphoric acid containing 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through

the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM

Retention time: 25
Internal standard: penicillin V (28.5)
Limit of detection: 1.4 ng/mL
Limit of quantitation: 2.0 ng/mL

OTHER SUBSTANCES

Extracted: ampicillin, cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS

derivatization; cow; SPE

REFERENCE

Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE

Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1

Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:10 mM pH 6.1 potassium phosphate buffer 5:95
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions
Sample preparation: Prepare amoxicillin sodium solutions in water adjusted to pH 4 with trifluoroacetic acid.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil ABZ+plus (Supelco)
Mobile phase: Gradient. A MeCN:0.1% pH 2.1 trifluoroacetic acid 7:93; B MeCN:0.1% pH 2.1 trifluoroacetic acid 20:80. A:B 100:0 for 13 min, from 100:0 to 40:60 in 2 min, maintain at 40:60 for 30 min, from 40:60 to 100:0 in 2 min, maintain at 100:0 for 13 min
Flow rate: 1
Injection volume: 5
Detector: UV 230; MS, PE-Sciex API I, ionspray interface at 5500 V, orifice potential voltage 50 V, m/z 100-800

CHROMATOGRAM
Retention time: 9.99

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Valvo,L.; Ciranni,E.; Alimenti,R.; Alimonti,S.; Draisci,R.; Giannetti,L.; Lucentini,L. Development of a simple liquid chromatographic method with UV and mass spectrometric detection for the separation of substances related to amoxicillin sodium, *J.Chromatogr.A*, **1998**, *797*, 311–316.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.85

OTHER SUBSTANCES

Also analyzed: antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Isolute SCX SPE cartridge (Jones Chromatography, Hengoed, UK) with MeOH and water. Condition a 100 mg PGC (porous graphitic carbon) SPE cartridge (Shandon, Runcorn, UK) with acetone and pH 7.7 borate buffer. Add 20 mL water to 5 g tissue, homogenize, add 5 mL 170 mM sulfuric acid and 5 mL 5% aqueous sodium tungstate, mix well, centrifuge at 14000 g for 5 min. Discard pellet, add six drops orthophosphoric acid to the supernatant to adjust the pH to 2-2.5. Add it to the SCX SPE cartridge, allow to flow through the cartridge under vacuum at 2 mL/min, wash with 5 mL 10 mM sulfuric acid, elute with 10 mL pH 7.7 borate buffer. Add the eluate to the PGC SPE cartridge. Wash with 5 mL water, place in-line filter (0.2 μm, Anotop) below cartridge, elute with 20 mL acetone. Evaporate to dryness. Add 500 μL water to the dry residue, add 20 μL 2% acetic anhydride in MeCN and let stand for 3 min. Add 500 μL triazole/mercuric chloride derivatizing reagent and heat the mixture at 65° for 20 min. Inject a 100 μL aliquot. (Borate buffer was 200 mM boric acid adjusted to pH 7.7 with 40% NaOH solution. Triazole/mercuric chloride reagent was prepared by mixing 34.45 g 1,2,4-triazole with 150 mL water and 25 mL 10 mM mercuric chloride, adjusted to pH 9.0 with 1 M NaOH and made up to 250 mL with water.) GL –5 μm Kromasil KR 100 C8 (Hichrom)

HPLC VARIABLES

Column: 250 × 3.2 5 μm Kromasil KR 100 C8 (Hichrom)
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM potassium dihydrogen phosphate and 15 mM sodium thiosulfate)
Flow rate: 0.55
Injection volume: 100
Detector: UV 325

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 5 μg/kg (muscle)
Limit of quantitation: 50 μg/kg (muscle), 100 μg/kg (liver)

OTHER SUBSTANCES
Extracted: ampicillin

KEY WORDS
SPE; cow; liver; muscle; derivatization

REFERENCE
Rose,M.D.; Tarbin,J.; Farrington,W.H.; Shearer,G. Determination of penicillins in animal tissues at trace residue concentrations: II. Determination of amoxicillin and ampicillin in liver and muscle using cation exchange and porous graphitic carbon solid phase extraction and high-performance liquid chromatography, *Food Addit.Contam.*, **1997**, *14*, 127–133.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak C18 SPE cartridge with 5 mL MeOH, 2 mL water, and 2 mL 2% trichloroacetic acid. Homogenize (Ultra-Turrax T25) 5 g blended tissue with 20 mL 10 mM pH 4.5 phosphate buffer at 10000 rpm for 1.5 min, centrifuge at 4500 rpm for 10 min, decant supernatant, homogenize residue with another 20 mL buffer, centrifuge. Combine the supernatants and filter them through glass wool, add 1 mL 75% trichloroacetic acid to the filtrate, vortex for 30 s, centrifuge at 4500 rpm for 20 min, filter the supernatant through glass wool. Add the filtrate to the SPE cartridge at 1-2 mL/min, wash with 2 mL 2% trichloroacetic acid, wash with 2 mL water, elute with 1.5 mL MeCN at 0.7 mL/min. Add the eluate to 500 μL water and 3 mL ethyl ether, vortex gently for 30 s, centrifuge at 2000 rpm for 3 min, discard the organic layer. Add 200 μL 20% trichloroacetic acid solution to the aqueous phase, vortex for 15 s, add 200 μL 7% formaldehyde in 400 mM citric acid, vortex for 30 s, heat in boiling water bath for 30 min, cool to room temperature, add 500 mg NaCl, mix briefly, add 3 mL ethyl ether, vortex for 1 min, centrifuge at 2000 rpm for 3 min, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, vortex thoroughly, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 S5 ODS2
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM KH_2PO_4 adjusted to pH 5.6 with KOH.)
Flow rate: 1 for 10 min then 2
Injection volume: 50
Detector: F ex 358 em 440

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.5 ppb (catfish), 0.8 ppb (salmon)
Limit of quantitation: 1.2 ppb (catfish), 2.0 ppb (salmon)

KEY WORDS
derivatization; fish; catfish; salmon; SPE

REFERENCE
Ang,C.Y.W.; Luo,W.; Hansen,E.B.,Jr.; Freeman,J.P.; Thompson,H.C.,Jr. Determination of amoxicillin in catfish and salmon tissues by liquid chromatography with precolumn formaldehyde derivatization, *J.AOAC Int.*, **1996**, *79*, 389–396.